1264457

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

December 20, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/519,800*
FILING DATE: *November 13, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/38021*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office

18351 U.S.PTO

Please Type a plus sign (+) inside this box → +

/SB/16 (02/01)
Approved for use through 10/31/2002. OMB 0651-0032
U.S Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

U.S.PTO 10/519800 12/30/04

111303

Express Mail Label No.: EV 333517073 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
William D. John	Hunt Cairney	Decatur, Georgia USA Atlanta, Georgia USA

☐ *Additional Inventors are being named on the first separately numbered sheet attached hereto.*

TITLE OF THE INVENTION (280 characters max)

DETECTION OF EUKARYOTIC AND PROKERYOTIC CELLS AND DNA USING A NANBIOSENSOR

CORRESPONDENCE ADDRESS

☐ Customer Number or Bar Code Label | (Insert Customer No. or Attach bar code label here) | Or ☒ Correspondence address below

NAME	Office of Technology Licensing Georgia Tech Research Corporation Attention: Director				
ADDRESS	505 Tenth Street, NW				
CITY	Atlanta	STATE	Georgia	ZIP CODE	30332-0415
COUNTRY	U.S.A.	TELEPHONE	404-894-6287	FAX	404-894-9728

ENCLOSED APPLICATION PARTS (*check all that apply*)

☒ Specification *Number of Pages* 33 ☐ CD(s), Number ____

☐ Drawing(s) *Number of Pages* ____ ☒ Other (Specify) Return postcard

☒ Application Data Sheet. See 37 CFR 1.76.

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27

☐ A check or money order is enclosed to cover the filing fees

☒ The commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 502064

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): 80.00

This invention was made by an agency of the United States government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,
GEORGIA TECH RESEARCH CORPORATION

SIGNATURE: Kimberly A. Dunn

TYPE or PRINTED NAME: Kimberly A. Dunn, Patent Coordinator

Date: November 13, 2003

Docket No.: 2799PR

REGISTRATION NO.:

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 USC 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time required to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Office, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

PATENTS
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Hunt, et al.

For: **DETECTION OF EUKARYOTIC AND PROKERYOTIC CELLS AND DNA USING A NANBIOSENSOR**

CERTIFICATE OF EXPRESS MAIL
FOR PROVISIONAL APPLICATION

Assistant Commissioner for Patents
Box PROVISIONAL PATENT APPLICATION
Washington, D.C. 20231

Sir:

Enclosed for filing in the above case are the following documents:

Provisional Application Patent Cover Sheet
Provisional Application
Fee Transmittal Form
Provisional Application Filing Fee, Small Entity - $80.00
Return Postcard

Respectfully submitted,

Kimberly A. Dunn
Patent Coordinator

GEORGIA TECH RESEARCH CORPORATION
OFFICE OF TECHNOLOGY LICENSING
505 Tenth Street, NW
Atlanta, Georgia 30332-0415

Our Reference No: **2799PR**

I hereby certify that all correspondences listed above are being deposited for delivery to the above addressee, with the United States Postal Service **"EXPRESS MAIL POST OFFICE TO ADDRESSEE"** service under 37 CFR §1.10 on the date indicated below:

The envelope has been given U.S. Postal Service "Express Mail Post Office To Addressee" Package # **EV 333517073 US**.

11-13-03
Date

Kimberly Dunn

A n vel Nan biosensor f r detecti n f cells, proteins and DNA

SUMMARY OF INVENTION:

Part I: a method to detect proteins in cell culture, the developmental stage of cells growing in culture and/or the production of specific proteins.

PartII: a method to detect the presence of bacteria or other microorganisms in liquid or gaseous phase. This would have applications in bioterrosism protection as such a device placed in water supplies or air ventilation channels would provide constant monitoring and a electrical early warning signal. The system could also have application in medical diagnosis and environmental monitoring.

Part III: a method using a Nanobiosensor to detect specific nucleic acid molecules. This sensor could have applications in pathogen detections, genotyping, gene expression assay, medical diagnosis and envirnonmental monitoring.

PART I: Developing Nanoacoustic Biosensors for Loblolly PineCell Culture and Mill Process Chemistry

Dr. John Cairney, Associate Professor, Forest Biology Group, Institute of Paper Science and Technology, Atlanta GA 30318

Dr. William D. Hunt, Professor, School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA 30332

EXECUTIVE SUMMARY:

Using Nanotechnology and Biotechnology, the objectives of this project are to develop an all-electronic, ultra-sensitive acoustic wave biosensor that will enable the detection of trace amounts of biomarker proteins in pine tissue culture and to demonstrate the potential of adapting this technology for Mill Process Chemistry. One of us (WH), using antigen binding to specific antibodies has developed a high frequency nanoacoustic sensor capable of detecting a few attograms of protein (1 attogram = 1 x 10 ^ -18 grams) in a gas-phase environment. This novel sensor technology will provide a new class of chips that can probe in real time a variety of target analytes. A chip (excluding the antibody biofilm) containing a 100 element array of 100μm x100μm sensors would cost roughly $3.50, further, a full system for reading the chip would be the size of a Palm Pilot or Blackberry Pager and hence would have a per-unit cost on the order of $500. Our experimental plan is to affix antibodies to a substrate and demonstrate the binding to them of plant cell proteins using the ultra-sensitive acoustic wave biosensor system, in a liquid phase environment. We will then use molecular biology procedures to clone a pine copy of the gene for an important marker protein (SERK), generate antibodies to this protein and using the above procedure, demonstrate detection of the protein in different cell lines, at the attogram level.

Interfacing Technology Areas: Project Description

This proposal describes work employing Biotechnology and Microelectronics at the nano-scale, to produce a novel highly-sensitive biosensor with molecular specificity. There are two main goals of the project:

1) Develop a tool to improve plant tissue culture. The NanoAcoustic biosensor (Nabio) will detect 'marker proteins' that serve to identify healthy pine embryogenic cultures. The project thus aligns with a program goal ('Develop a Higher Value Raw Material Supply') and with a current IPST program goal.

2) Build a platform for innovation in other areas of the industry. An approach, similar to that described below, has been demonstrated successfully by one of us (WH). By showing success in the pilot project described in our proposal, the technology can be expanded and adapted for other application areas to detect trace amounts of other small molecules in other forestry, pulp or paper making processes such as Oxalate or Barium Sulfate, using substrate interactions rather than Antigen-Antibody binding.

Industrial Need: The principal cost of paper making is raw material and the availability of low-cost fiber from fast growing trees in subtropical regions has given companies in South America and Southeast Asia a competitive advantage over US companies. Under the best management practices, elite trees grown in the Southern US match growth rates for subtropical regions. Somatic Embryogenesis, a plant tissue culture technique for pine embryo multiplication, has great potential of providing large numbers of elite seedlings (Pullman et al 1998, Grossnickle and Sutton, 1999). The process works well for spruce and fir and is in pilot commercial phase in Canada, however for loblolly pine, the most important tree in the Southern US, the process is recalcitrant and dependent on the genetic makeup of the source trees. Recent work by one of us (JC,Cairney et al 2000) shows that it is possible to identify the molecular differences between healthy embryo cultures and cultures that produce few or sickly embryos. At IPST we are conducting work to identify the key enzymes that will indicate embryo quality. Recent work in other laboratories has identified a Somatic Embryogenesis Receptor Kinase (SERK) protein whose activity stimulates embryogenesis (Hetch et al 2001). Identifying cultures with elevated levels of this protein or identifying treatments that boost these levels would be of great advantage to our program. A system for simple, rapid detection of key protein markers in real time would be a valuable tool in tissue culture.

Novel Strategy for Quantifying small amounts of Protein

To provide a brief introduction to our approach, we have constructed vapor phase biosensors by immobilizing a monolayer of antibodies onto the surface of a Surface Acoustic Wave (SAW) device as is shown schematically in Appendix, Figure 1.a. The device is then connected into an oscillator circuit and the frequency of oscillation can then be precisely measured. Frequency is the physical parameter which can most accurately be measured and resolution in the range of parts per billion are readily achieved. When the antigen binds to the antibody, the acoustic velocity is decreased and the oscillator frequency shifts to a lower value. For our devices we have obtained sensitivities of approximately 20Hz/pg with a detection limit just under a picogram.

Commercial Opportunity

Industrial Benefit. The potential of Somatic Embryogenesis for the Pulp and Paper Industry is great. An economic model developed by an industry consulting firm, Jaakko-Pöyry, was used to identify a wood fiber cost ranging from $100-140/FMT (finished metric ton product). Given a potential savings of $15/FMT (a consensus figure) on a US industry-wide production of 50 million wet tons softwood processed/year, the savings realized by production of seedlings through somatic embryogenesis would be about $1 billion/year. This figure does not include advantages that accrue from multiplication of genetically improved trees produced by breeding programs, thus savings may be even greater. The proposal focuses on performance of Somatic Embryo cultures, however the expansion of Nabio's, to the detection of compounds affecting product quality in the Mill is held in mind and this project intends to provide a delivarable of a new cell culture analysis tool and to create a platform for the expanding the use of that tool.

Federal Funding Opportunities for this Project. Considerable Government funding for this area of research is available. Approximately $46 million has been planned for NIRT and NER awards for this program solicitation in FY 2002, subject to availability of funds. The estimated Award size will be in the range $250,000 - $500,000 per year for up to four years (http://www.nsf.gov/pubs/2001/nsf01157/nsf01157.html). This commitment to Nanotechnology research is expected to continue. The preliminary work done in the course of the proposed project would permit submission of an NSF grant.

Project Elements, Deliverables, and Timeline

The project has two phases;

1) Demonstration of the ability to detect pine proteins in cell extract using this technology

2) Detection of pine SERK protein in pine cell culture

Objective 1: *Demonstration of the ability to detect pine proteins in cell extract.* We will focus, initially, on proteins known to be expressed in pine cell culture and employ antibodies to horseradish peroxidase. These antibodies detect proteins from a wide variety of species (Sigma and J.Dean, UGA, personal communication). In addition antibodies against GUS protein and NPT-11 protein may be used with transgenic pine cultures Antibodies to horseradish peroxidase and will be obtained commercially and fixed to a substrate according to previous practice (see Appendix). Cell culture media containing actively growing pine embryogenic cultures will be assayed. In addition early stage embryos will be harvested and cell extracts made. The detection system will be exposed to the media or cell extract and the binding of horseradish peroxidase will be detected. Since the Nabio sensor acts in real time reaction kinetics of the binding event can also be monitored.

Objective 2a *Isolation of pine SERK clone and generation of Antibodies.* Based on the published sequence of the Arabidopsis SERK protein, degenerate primers will be designed and a pine homolog will be cloned by PCR.. The PtSERK cDNA will be cloned into a protein expression vector and protein will be generated Antibodies will be generated in rabbits by a Animal Pharm Services Inc. (http://guide.labanimal.com/company/21.html).
Objective 2b Detection of SERK protein in pine tissue culture will be as described above 1.

Task	J	F	M	A	M	J	J	A	S	O	N	D
1. Obtain Peroxidase Antibodies and Fix to Substratum	X	X										
2. Conduct initial detection of Peroxidase using Nabio (proof of concept - general)		X	X	X	X							
3. Obtain pine SERK using PCR		X	X									
4. Express PtSERK and Isolate protein			X	X	X							
5.Generate PtSERK antibodies						X	X	X	X	X	X	
6. Set up Nabio with antibodies against PtSERK									X	X	X	
7. Assay cultures for PtSERK (proof of concept - specific)										X	X	X
8. Write and submit Grant Application										X	X	X
9. Write and submit article to scientific journal										X	X	X

References:

1. Cairney J, Xu N, Mackay J, Pullman J. 2000. Transcript profiling; A tool to assess the development of conifer embryos. In Vitro Cellular & Developmental Biology - Plant. 36(3): 155-162.
2. Grossnickle SC, Sutton BCS. 1999. Application of biotechnology for forest regeneration. New Forests 17: 213-226.
3. Hecht V, Vielle-Calzada J-P, Hartog MV, Schmidt EDL, Boutilier K, Grossniklaus U, de Vries SC. 2001. The Arabidopsis Somatic Embryogenesis Receptor Kinase 1 Gene Is Expressed in Developing Ovules and Embryos and Enhances Embryogenic Competence in Culture. Plant Physiol. 2001 127: 803-816.
4. Pullman GS, Cairney J, Peter G. 1998. Clonal Forestry and Genetic Engineering: Where we stand, future prospects, and potential impacts on mill operations. TAPPI J. 81(2): 57-64

APPENDIX t Cairney and Hunt –Innovation 2002

Developing Nanoacoustic Biosensors for Loblolly Pine Cell Culture and Mill Process Chemistry

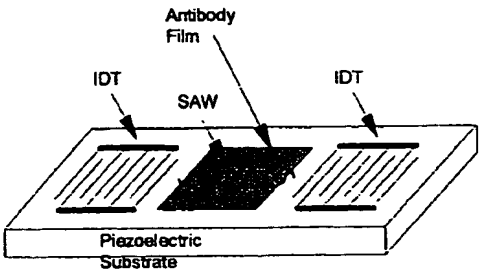
Dr. John Cairney, Associate Professor, Forest Biology Group, Institute of Paper Science and Technology, Atlanta GA 30318

Dr. William D. Hunt, Professor, School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA 30332

Novel Strategy for Quantifying small amounts of Protein

To provide a brief introduction to our approach, we have constructed vapor phase biosensors by immobilizing a monolayer of antibodies onto the surface of a Surface Acoustic Wave (SAW) device as is shown schematically in Figure 1a). The device is then connected into an oscillator circuit and the frequency of oscillation can then be precisely measured. Frequency is the physical parameter which can most accurately be measured and resolution in the range of parts per billion are readily achieved. When the antigen binds to the antibody, the acoustic velocity is decreased and the oscillator frequency shifts to a lower value. For our devices we have obtained sensitivities of approximately 20Hz/pg with a detection limit just under a picogram.

To verify vapor phase analyte binding events, we developed a fluorescent antibody/analyte assay. This was to provide a method, independent of the SAW device response, to detect the occurrence of a molecular binding event. SAW devices with and without anti-FITC antibody films were tested against two different fluorescent analytes: uranine and Alexa Fluor®. A specific analyte was presented by bubbling nitrogen at 1ml/sec through one liter of a 1nM aqueous solution of the analyte compound. After brief, 15 second, exposure to the analyte vapor in the SAW sensor system, the TO-8 packaged devices (Figure 1b) were pulled from the system, washed with buffer to remove unbound analyte, and then viewed using a Zeiss LSM510 confocal fluorescent microscope (CLSM). If fluorescence was observed in the CLSM image, this was taken as evidence of bound fluorescent analyte.



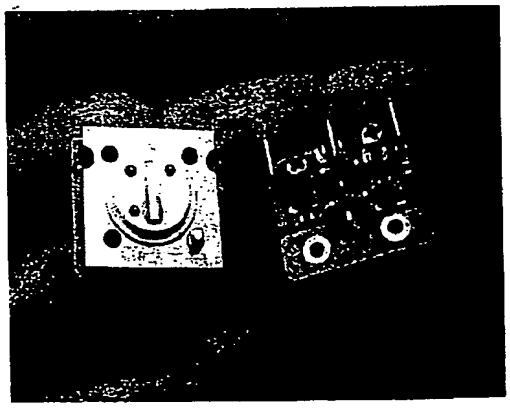
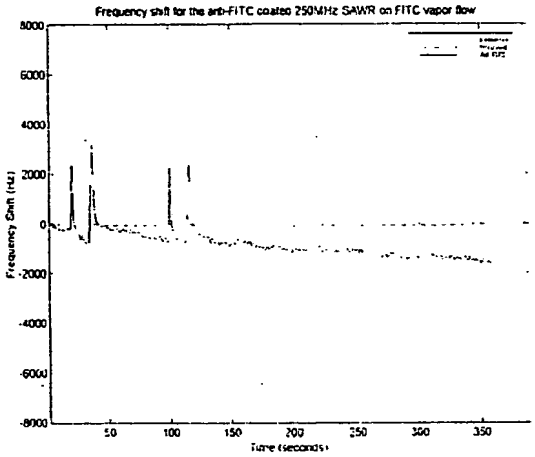


Figure 1: a)SAW Immunoassay Sensor b)TO-8 Packaged SAW resonator and attendant oscillator circuit

Our technique for ST-Quartz SAW resonator biosensors[Stubbs, 2001 #9] will soon be published in Biosensors and Bioelectronics. By using fluorescent molecules as the analyte, Hunt was able to observe the molecular recognition event by two independent means: fluorescent microscopy and resonator frequency shift. Fluorescent spots in the CLSM image, Figure 2b, shown below represents the uranine molecules captured by the anti-FITC antibodies immobilized on the metal electrodes. These fluorescent molecules were not released even after washing with a buffer, handling and a time period of roughly 30 minutes. In our methods we used a thin layer of hydrogel over the immobilized antibodies to provide a near-aqueous environment necessary for maintenance of the tertiary structure of these biomolecules. The electronic measurements, shown in Figure 2a), indicate the binding of the antigen to the antibody by virtue of a baseline shift in the SAW resonator response. This work demonstrates that an acoustic based biosensor can be used for the detection of small molecules in the vapor phase.

In the proposed project we will extend this work for a more conventional immunoassay application, namely the detection of proteins in the liquid phase. We will immobilize anti-SERK antibodies onto the surface of high frequency 100μm diameter ZnO resonators which are capable of sensitivities in the attogram range. The surface displacement of the sensor is in the range of a few Angstroms and the antibodies are nanoscale structures specifically designed for molecular recognition. We will tailor our designs in this program for low-cost, ultra-sensitive applications for embryonic cell cultures.



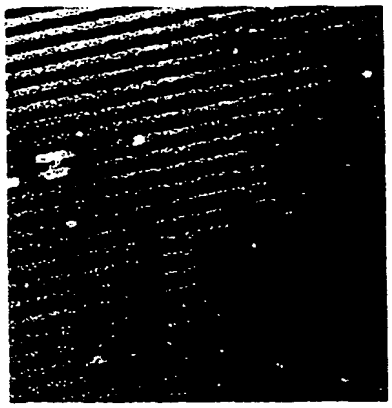


Figure 2: a) SAW response to uranine

b) CLSM image of anti-FITC SAW chip after exposure to uranine vapor

Hunt's group has done considerable work in the area of fabrication, testing and analysis of acoustic waves in ZnO thin films on GaAs and will leverage that expertise in this program [Kim, 1994 #23][Kim, 1995 #26][Smith, 1995 #25][Kim, 1995 #26][Olutade, #27]. A photograph of one of these acoustic resonators that we have fabricated on a substrate which also contains GaAs HBT electronics is shown in Figure 4.a. This picture represents some of the results of an ongoing project between RF Micro•Devices (RFMD) of North Carolina and Georgia Tech. We have also deposited ZnO films using the same RF Magnetron sputtering system for the creation of high-frequency (1.9 GHz) resonators on Si substrates. These ZnO thin film

resonators (TFRs) are comprised of a thin, *e.g.* 1.6μm, layer of ZnO sandwiched between two metal electrodes. This ZnO film structure lies over a via hole micromachined through the GaAs substrate.

There are several aspects of our accomplishments to date in ZnO deposition which have a substantial impact on the proposed project. We have been able to demonstrate ZnO film thickness uniformity of less than 1.5% over a 3 inch wafer with piezoelectric coupling constant, k_t, which is 93% of the value predicted for pure crystalline ZnO. In addition, we have typically achieved resonator Qs of 450 for TFRs these films on Si substrates. In short, we have demonstrated that we can sputter high quality ZnO films over a large substrate area which is absolutely essential for the proposed 2D biosensor array.

Potential Uses of Invention Described in Part I:

Detection of proteins produced by plant, animal or yeast cells.

These proteins may act as indicators to reveal the developmental stage of cells growing in a bioreactor or the production of desired proteins by such cells

Use in Bioreactors

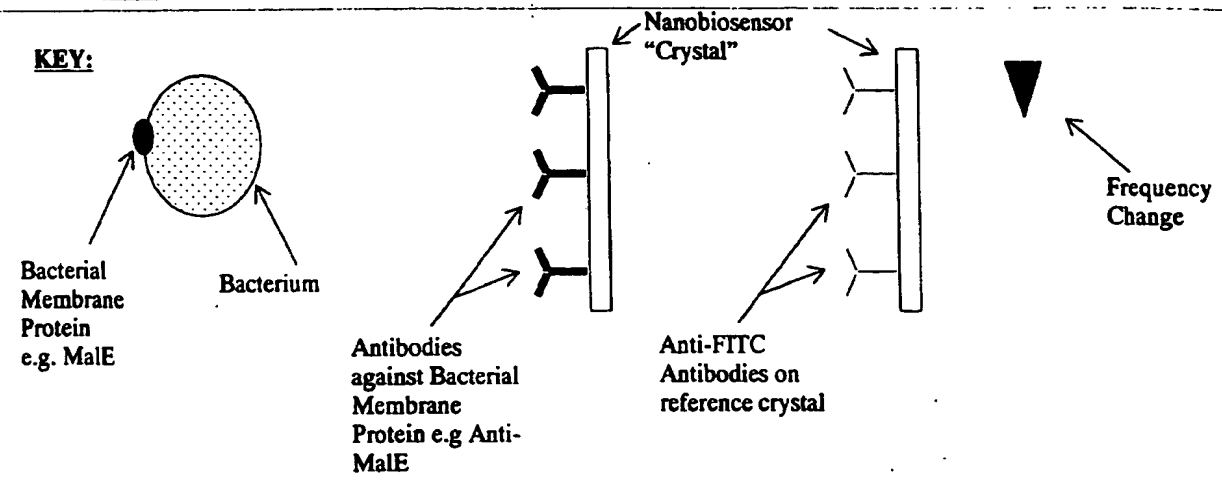
The ability to reflect the presence of a specific protein by an electrical signal can find use in process cell culture, biochemistry and chemistry, since the system could be automated such that additions could be made to a reaction in response to the electrical signal generated when the protein is detected. This has applications in process control.

This system could be adapted to detect molecules other than proteins using the same process.

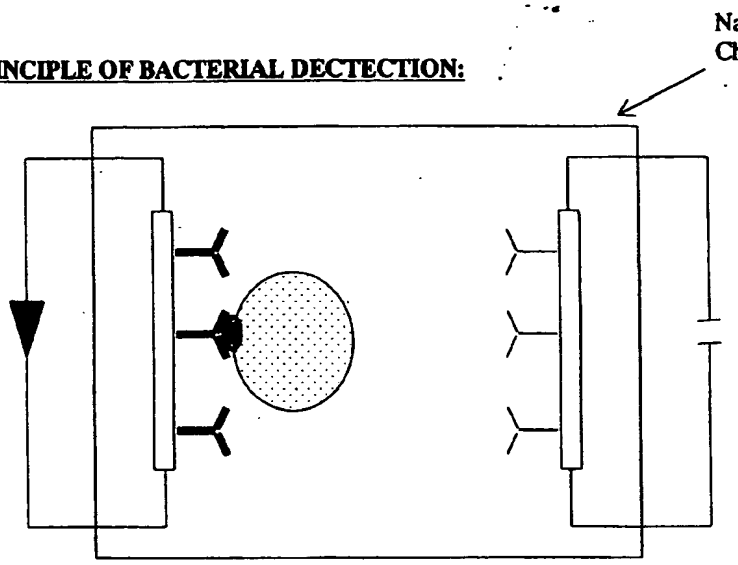
The density of a culture could be monitored using the same system, where a culture was heterogeneous and only a certain form of cell (a certain stage of development or growth) was of interest, the number of cells of that particular form could be detected above a background of similar but undesired cells. Such a discrimination would be difficult or time consuming using current methodologies.

PART II: Detection f Bacteria Using a N vel Nan biosensor

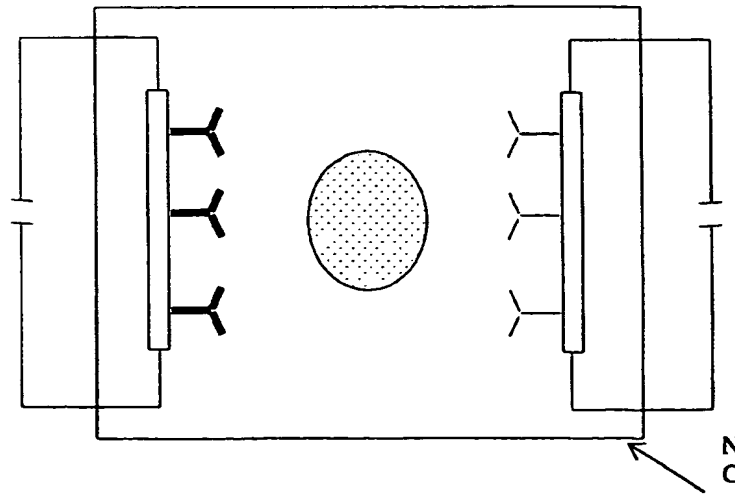
KEY:



PRINCIPLE OF BACTERIAL DECTECTION:



Bacteria expressing a membrane protein are bound to crystal via a binding event between a bacterial membrane protein (e.g. MalE) and an antibody which specifically recognizes that membrane protein (in this example, anti-MalE). A frequency change occurs in the nanobiosensor.



Bacteria NOT expressing the specific membrane protein recognized by the antibodies on the crystal are NOT bound and no frequency change results.

Potential Uses of Invention Described in Part II:

Safety/Bio-terrorism:

The presence of a Nanobiosensor in streams, water supplies or any other desired location would be of great values as real time, instant electronic signals can be detected to reveal the presence of a targeted probe.

The presence of such a Nanobiosensor in air cooling systems and other environmental conditioning systems would provide instant messaging and continous monitoring of air supply. The value of swift alert to building occupants could result in the saving of lives.

The presence of a particular form of a microorganism (e.g the virulent form of pathogen) could be detected via the nanobiosensor detecting a microbial protein associated with that state. In this way the sensor can detect modifications in microbial gene expression which are of interest to the person monitoring the system.

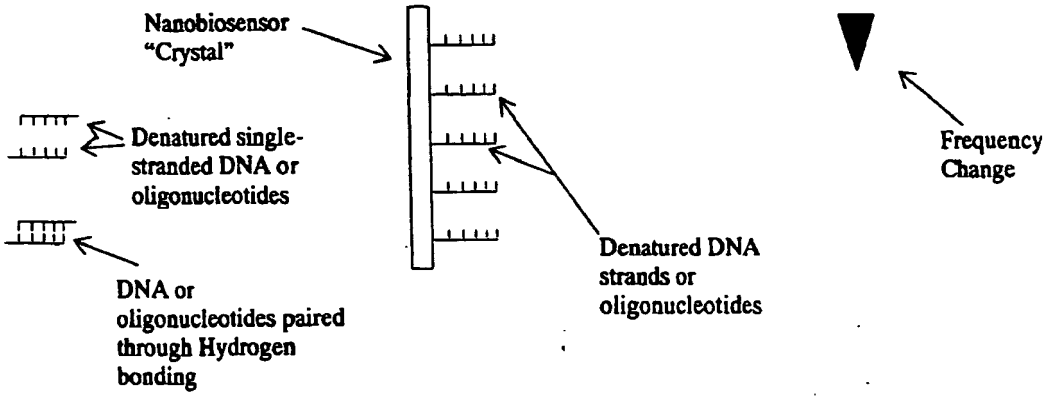
Medicine:

The detection of pathogens in patients in bodily fluids (e.g. blood)

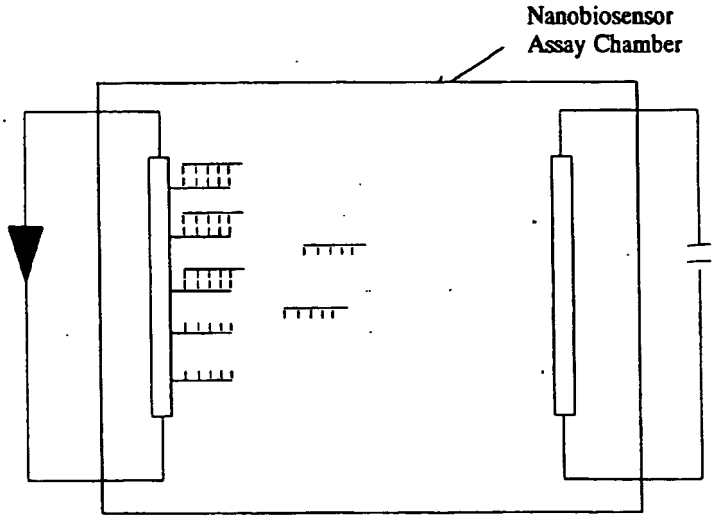
The detection of pathogens in patients in bodily fluids through implantation adjacent to indwelling medical devices

PART III: Detection of DNA molecules using a N vel Nanobiosensor

KEY:



PRINCIPLE OF DNA DECTECTION:



DNA hybridizing to specific sequences tethered to the crystal will produce a frequency change. Non-homologous DNA will not bind and no frequency change will occur.

Potential Use of the Invention Described in Part III:

Gene Expression Detection;

1. This biosensor can find use in DNA arrays, DNA chips and similar gene expression assay systems where nucleic acid hybridization is used as an assay of gene expression. This system would produce an electrically detected signal, revealing DNA hybridization to the DNA coated on the biosensor. In this respect the biosensor is similar to one element of a DNA array. DNA array hybridization currently is detected using imaging technology: binding of fluorescently- or radioactively-tagged probes is detected and intensity of bound probe is quantified and often viewed as 'false' color. Detection occurs after hybridization signals are processed. The Nanobiosensor described above would provide instantaneous information with the amount of hybridization (and thus amount of a specific nucleic acid molecule) reflected in the scale of frequency change.

   An array of 10 000 nanobiosensors, each coated with a specific nucleic acid molecule, would give rapid information on 10 000 separate hybridization reactions. In a system where individual Nanobiosensor outputs could be separately monitored expression of specific genes could be followed and instant information on gene expression could be obtained.

Medicine:

Monitoring patients responses to drugs
Monitoring patients progress during illness
Early detection of physiological responses – an aid in diagnosis

Agriculture:

Monitoring plant responses to environmental changes or to application of nutrients.

Environmental Monitoring:

Monitoring changes in microorganisms, flora or fauna in streams, wetlands etc

DNA Genotyping:

The biosensor would find use in detection of specific genetic traits through selective hybridization to different DNA sequences. Specific alleles, or mutations or single nucleotide polymorphisms (SNPs) can be detected by hybridization. The Nanobiosensor described above would provide instantaneous information on hybridization.

This invention would find use in:

Genetic Screening:

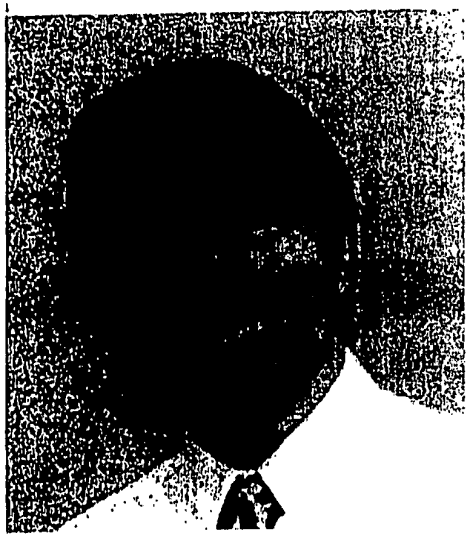
Early detection of sequences linked to genetic diseases or predisposition could be detected as outlined in example1 above.

Microorganisms, viruses etc could be detected by virtue of hybridization. This would find application in both medical fields and environmental monitoring as human and/or animal or plant pathogens could be rapidly detected.

Safety/Defense against Bio-terrorism;

The presence of a Nanobiosensor in streams, water supplies or any other desired location would be of great values as a real time, instant electronic signal can be detected to reveal the presence of a targeted probe.

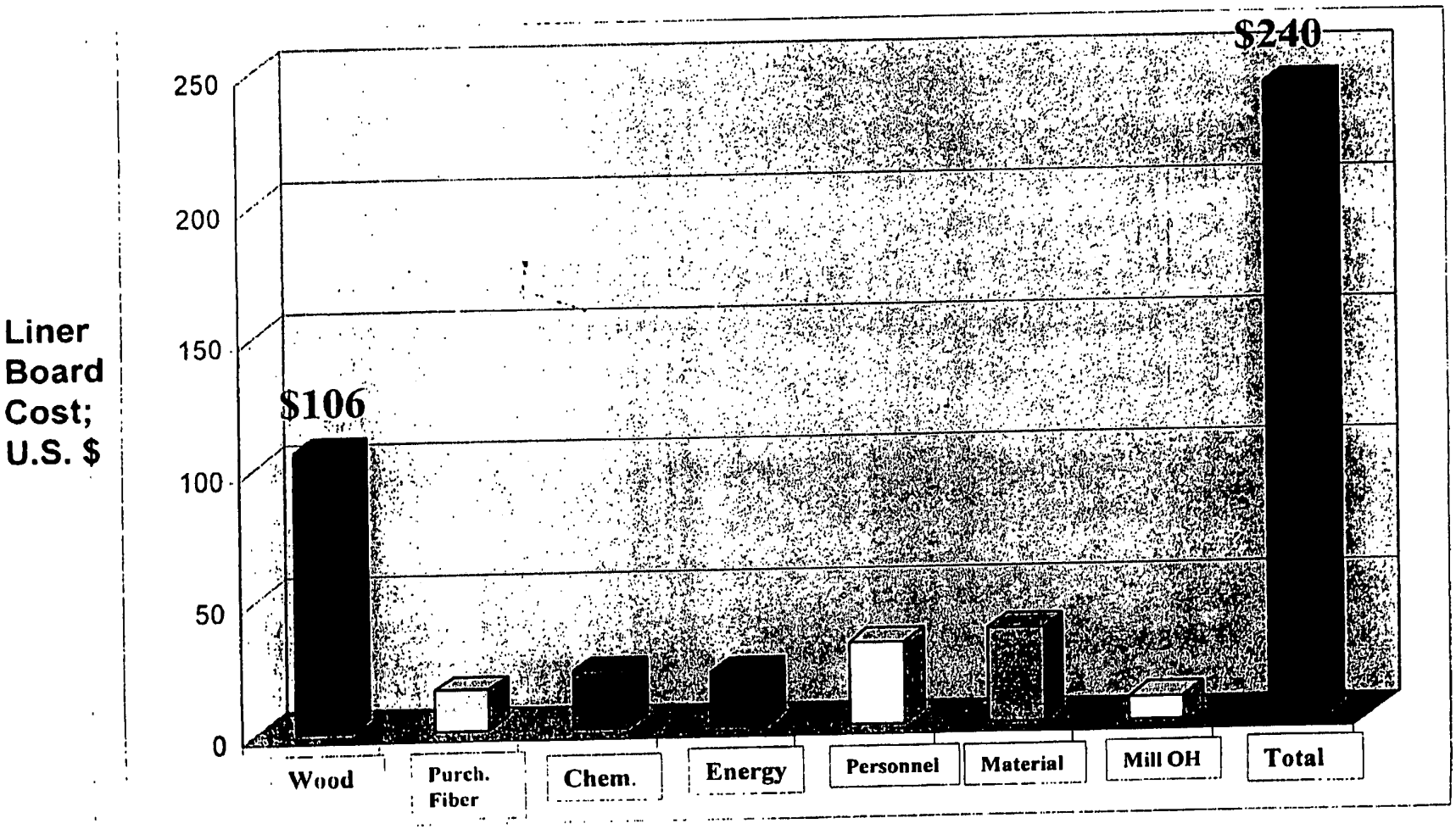
Nanoacoustic Biosensors for Loblolly Pine Cell Culture and Mill Process Chemistry Applications



Dr. John Cairney
Associate Professor, IPST

Dr. William Hunt
Professor, GT

Wood Is the Principal Cost Component of Papermaking



Source: IPST Economic Model

Rate of Tree Growth in South America and Southeast Asi Confers an Economic Advantage on These Countries

Pines grow three times faster in subequatorial countries than in the U.S.

Pinus radiata **40 $m^3\ ha^{-1}yr^{-1}$ New Zealand**

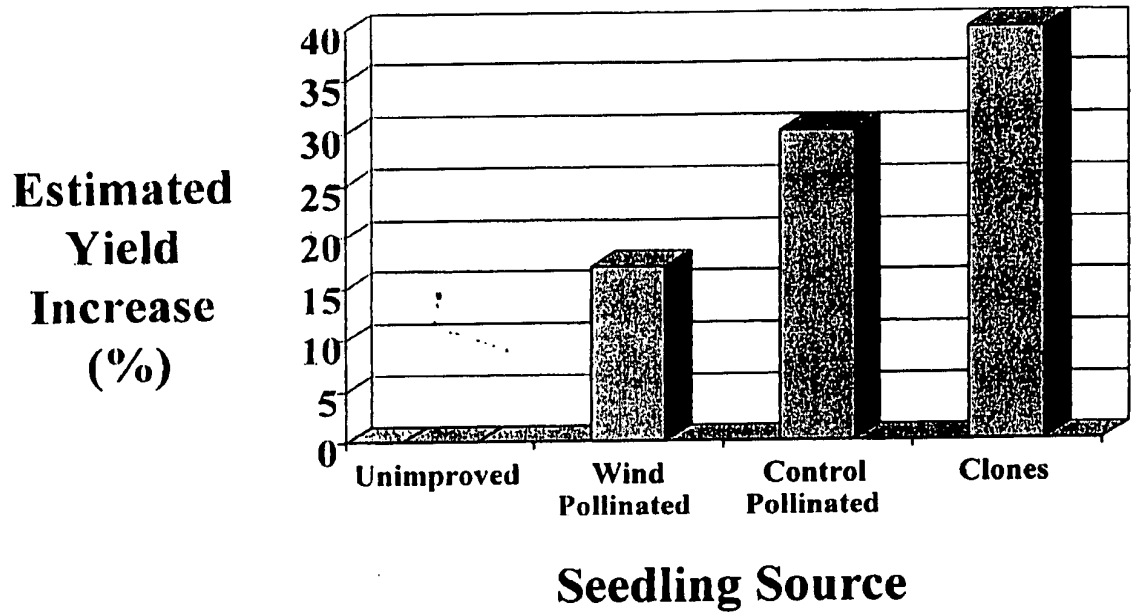
Eucalyptus hybrids 70 $m^3\ ha^{-1}yr^{-1}$ South America

In the last decade, U.S. import of forest products grew three times faster than exports

Net increase in the U.S. Forest Product trade deficit; grew from $1.6 billion (1990) to $ 8.3 billion (2000)

(FAO-UN, http://apps.fao.org/page/collections?subset=forestry)

Clonal Propagation Will Increase Forest Yield



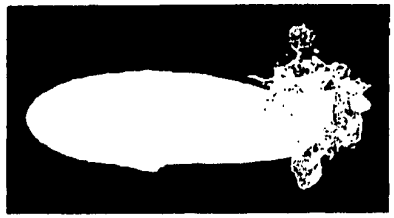
Somatic Embryogenesis:

A clonal propagation method

- **Captures the best qualities of trees**

Can produce > 1000 trees from one seed instead of one tree per seed

Initiation 1-57%



100/16

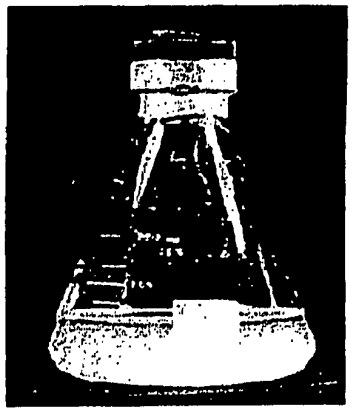
Capture 6-60%

Conversion 80-100%

2/2

Fiber Supply: Clonal Propagation Through Somatic Embryogenesis Has Great Potential But Is Inefficient

16/3



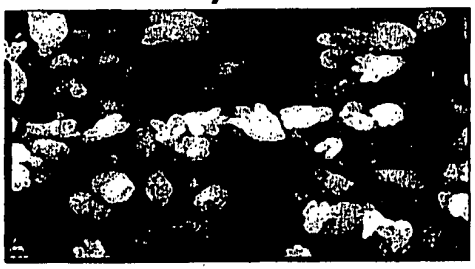
Multiplication 100%

Germination 3-80%

2/3

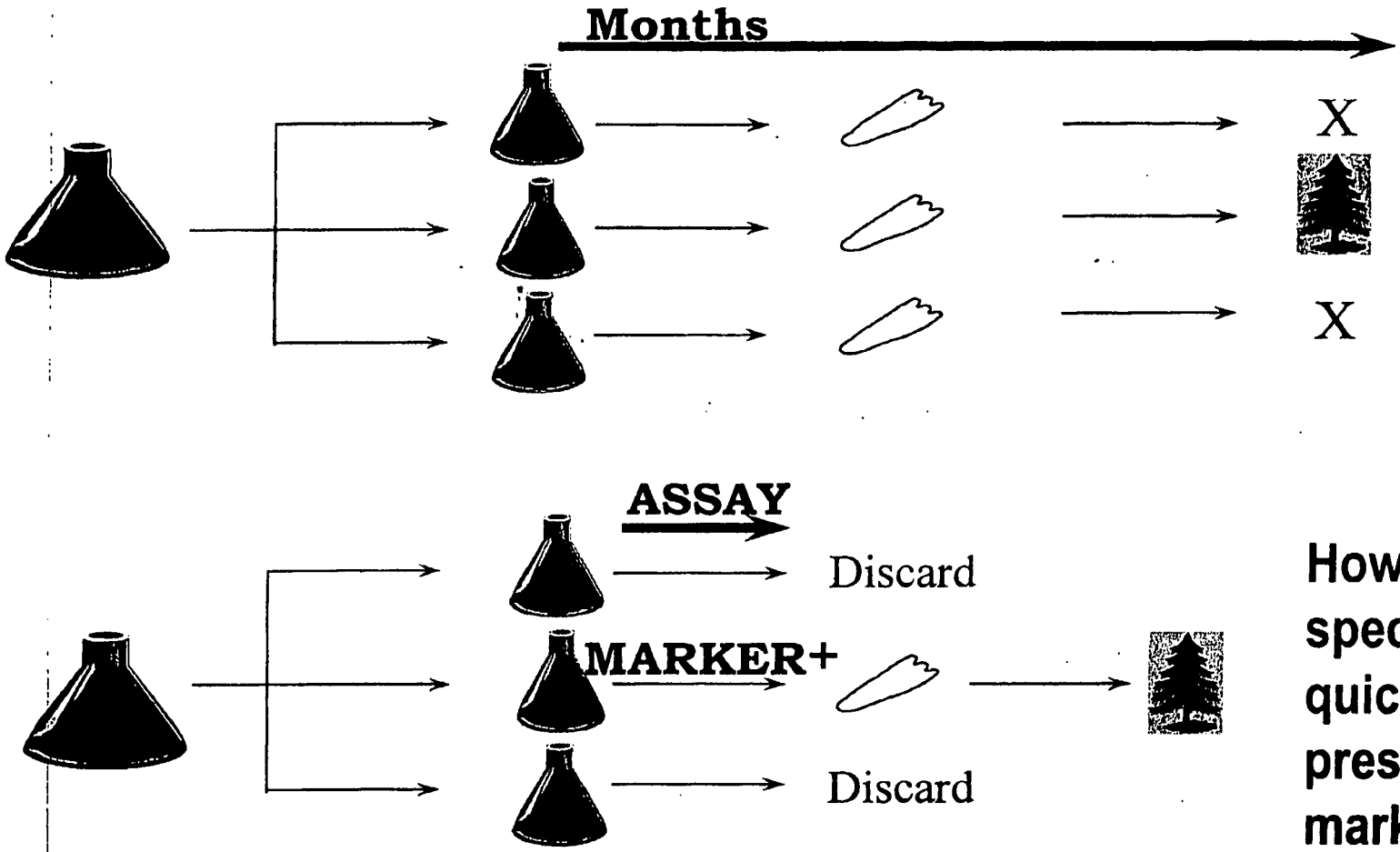
3/3

3/3



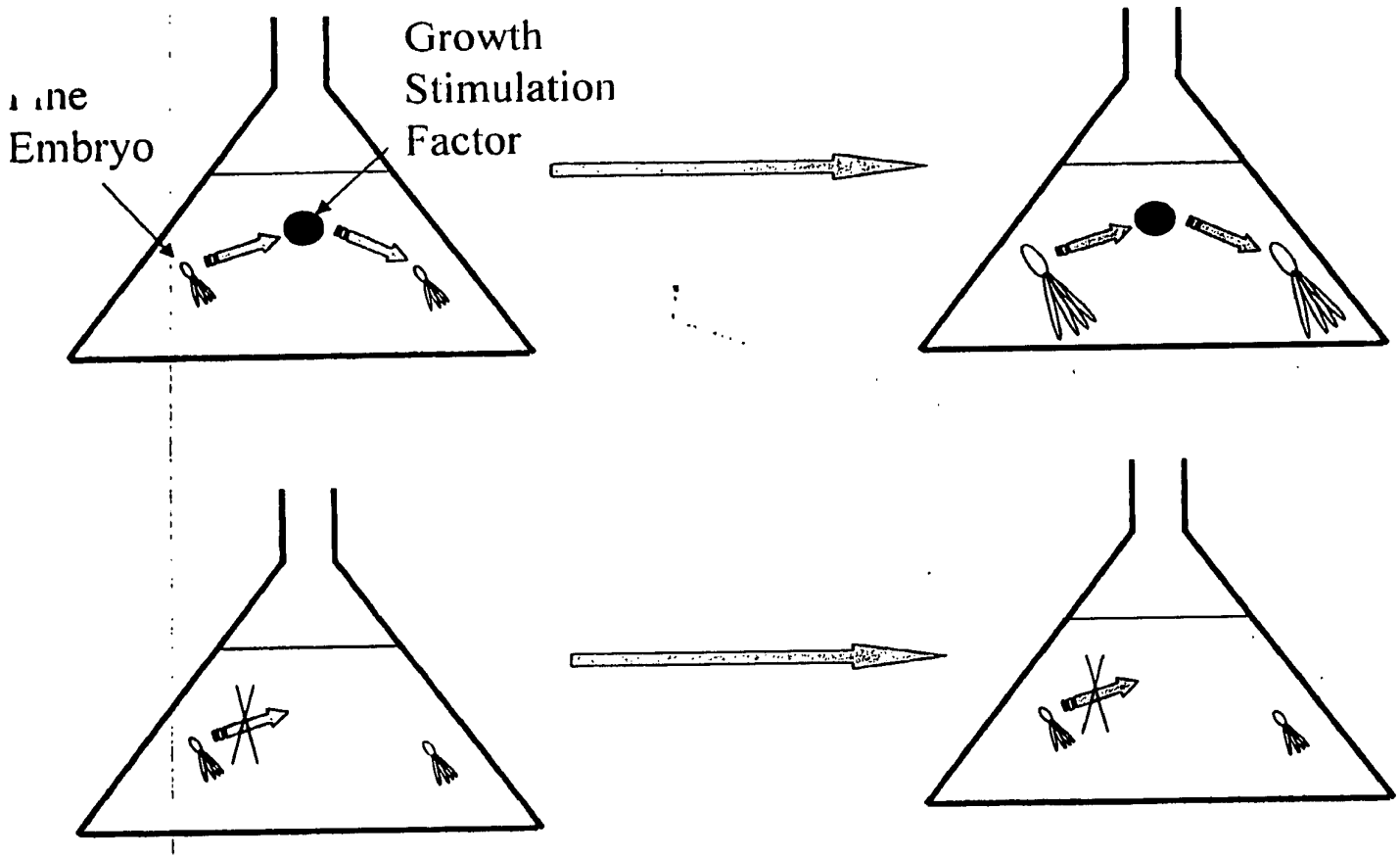
Maturation 5-90%

Identifying Cell Lines with Strong Embryogenic Potential Would Save Time and Money



How can you specifically and quickly detect presence of marker?

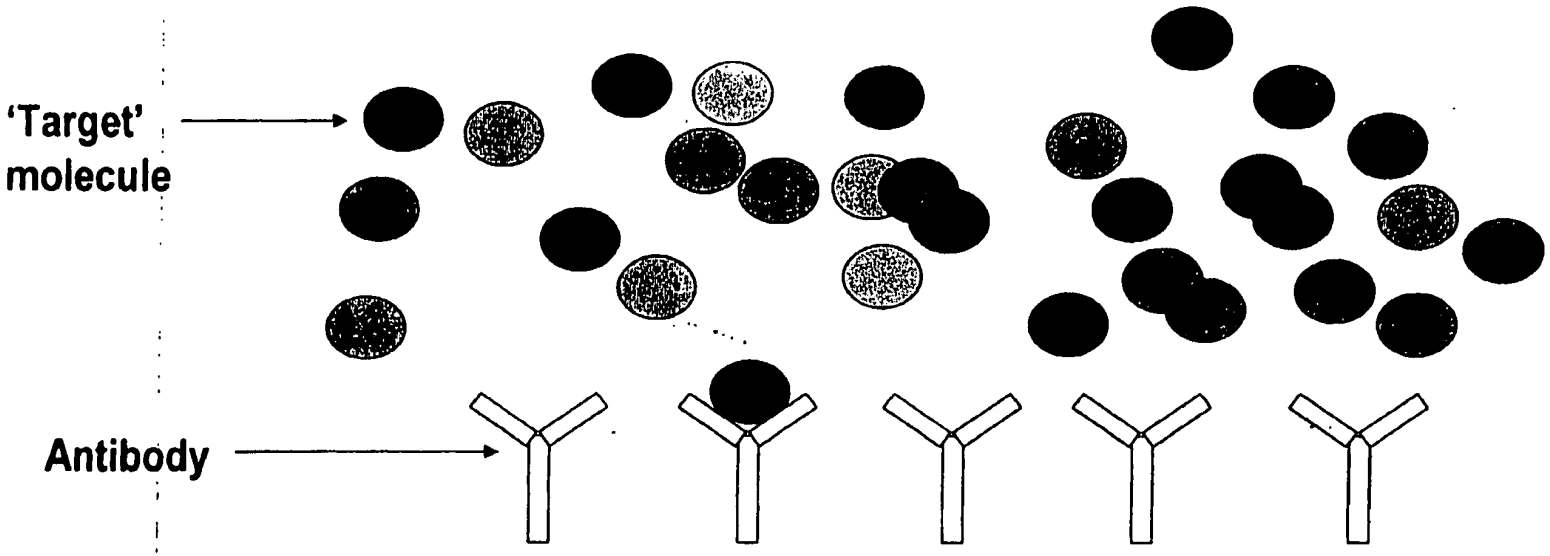
Factors Secreted by Embryos Stimulate Growth



Some Factors (Markers) are now known; others are being identified

How can you specifically and quickly detect presence of marker?

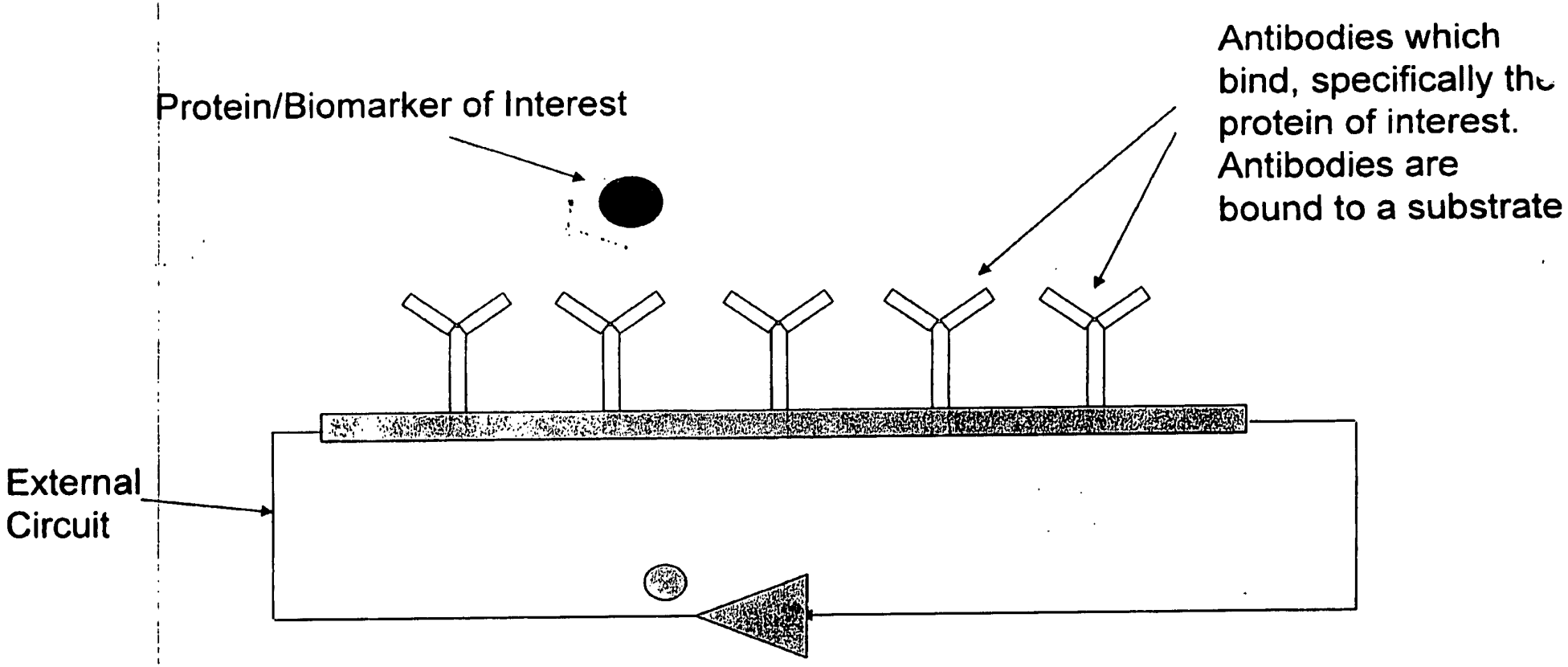
Antibodies are Molecularly Specific and Bind Targets in a Discriminating Fashion



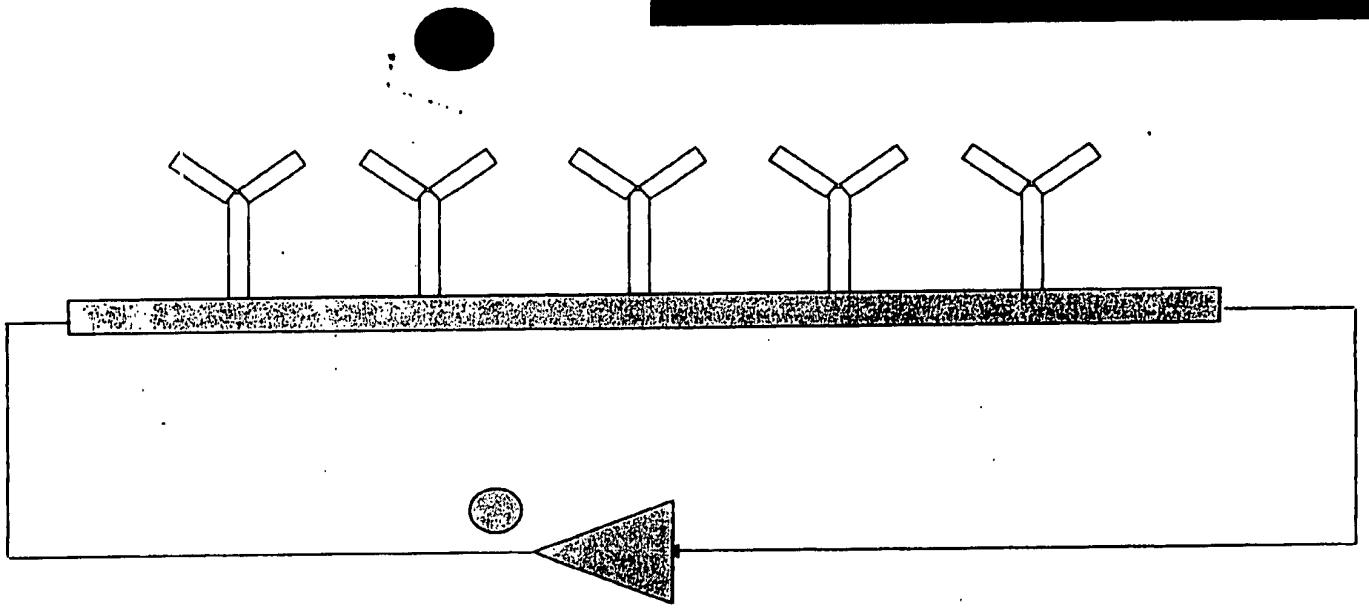
Antibodies can be raised against essentially any target

Conventional antibody binding assays require several hours

Nanobiosensor Principle: By Coupling Antibodies to a Surface Acoustic Wave (SAW) Resonator, Antibody Binding Is Detected Instantly

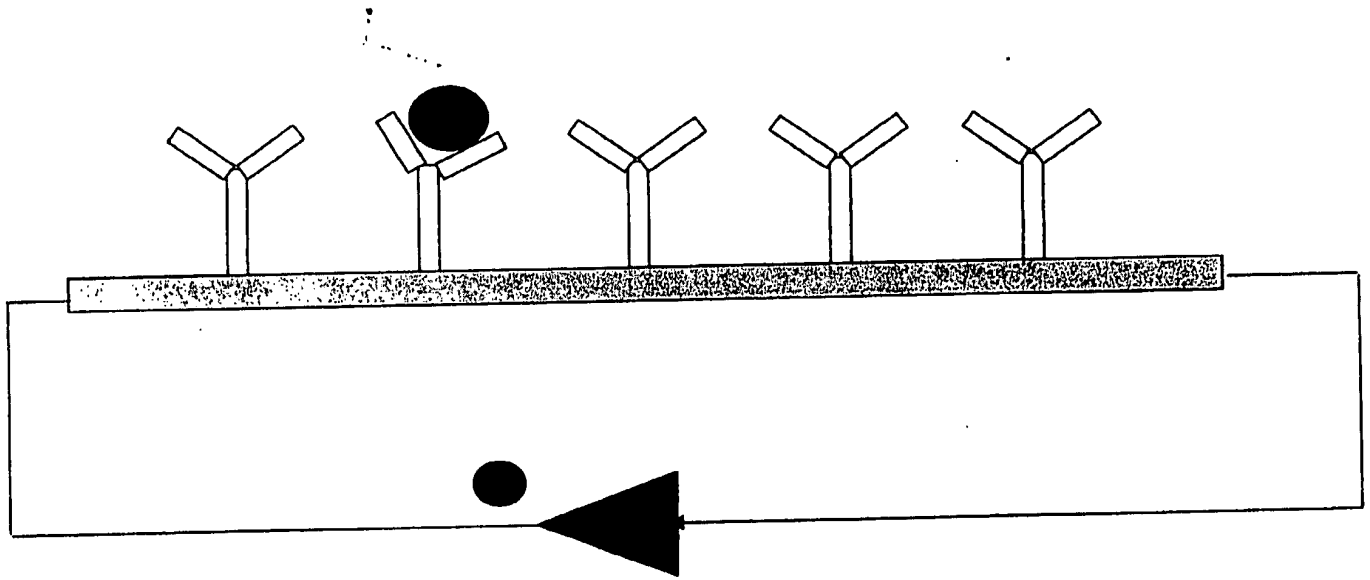


Nanobiosensor Principle

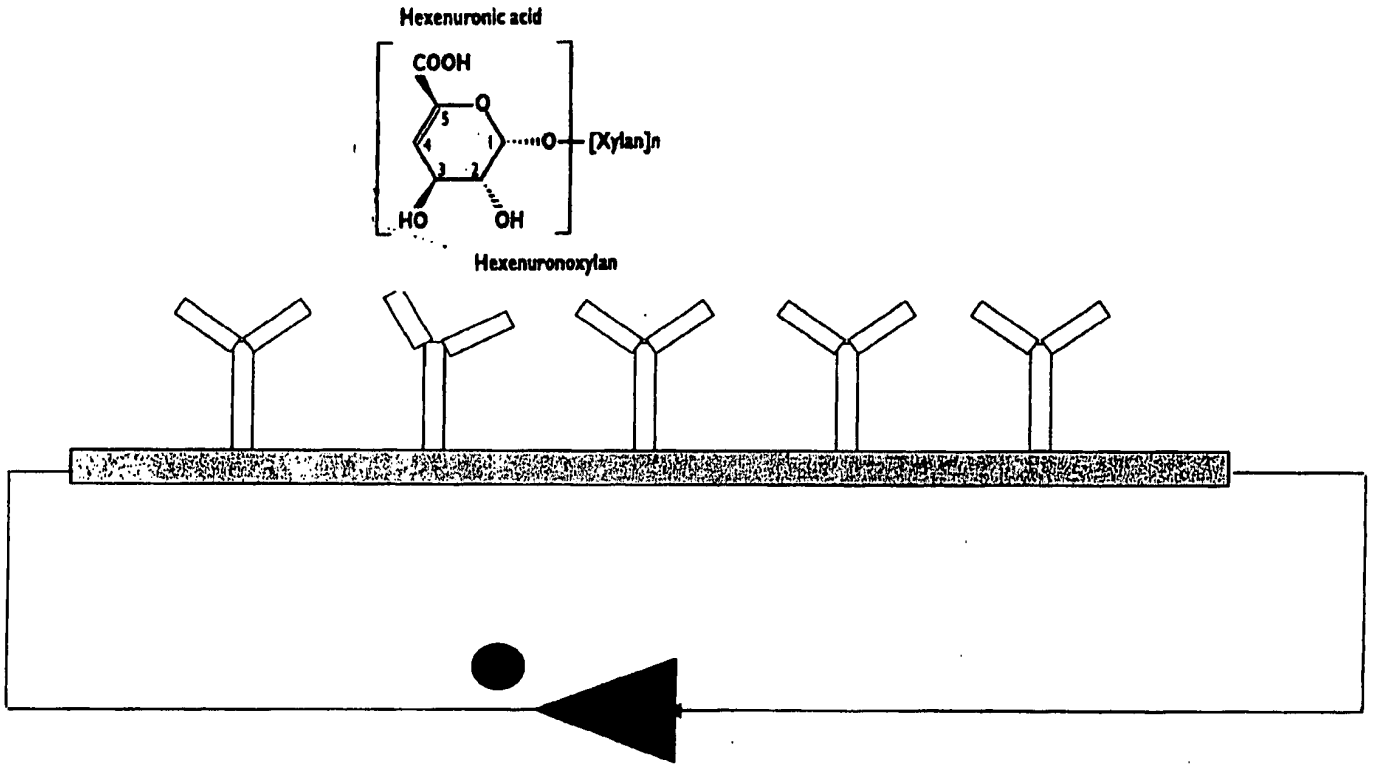


Nanobiosensor Principle

Binding of protein causes change in antibody conformation resulting in electrical signal



Nanobiosensor Principle: Simple Organic Molecules Can Also Be Detected



Sauerbrey Equation

Sensor resonant frequency

Frequency shift

Mass change

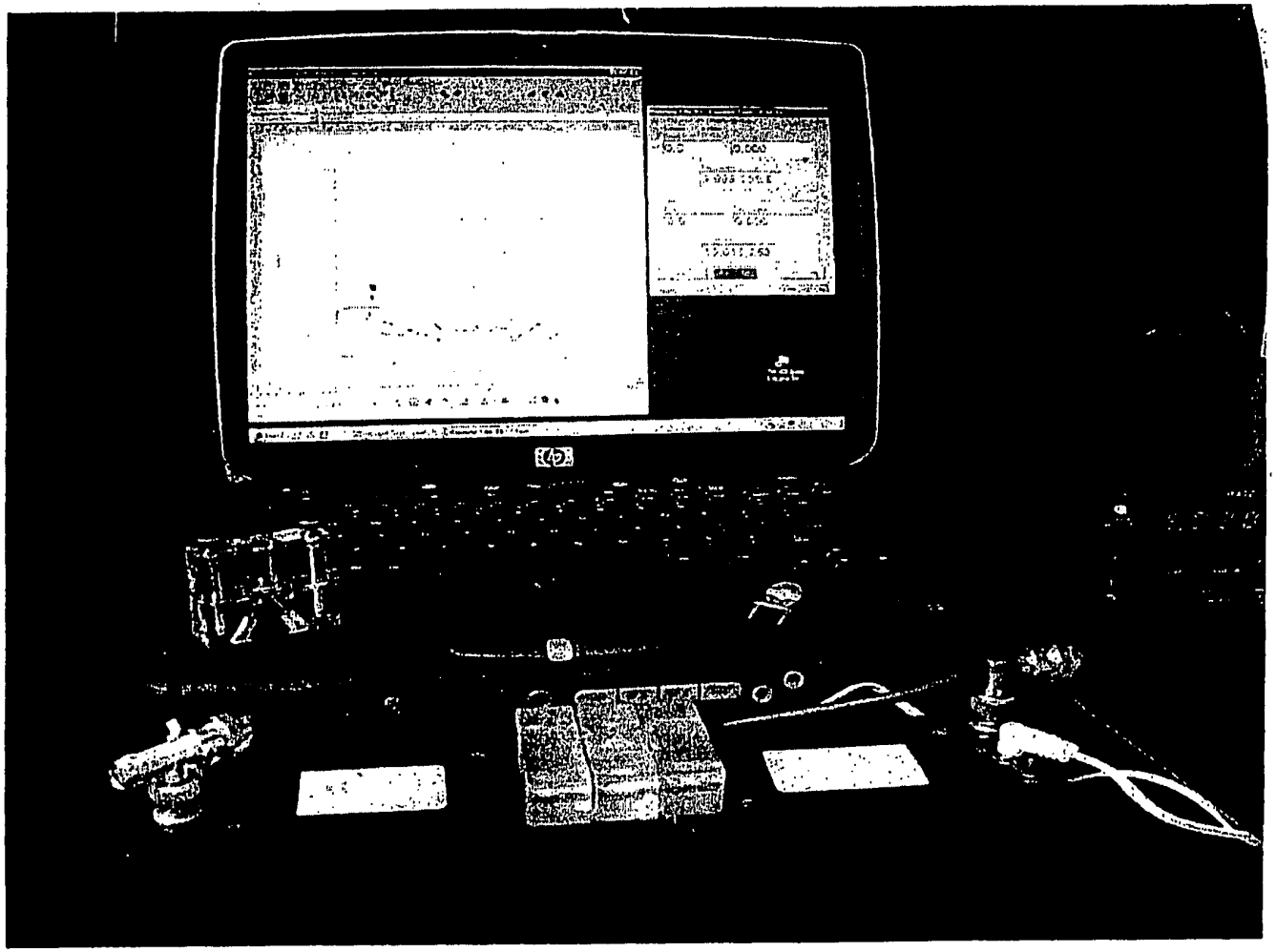
$$\Delta f = \frac{-2 f_o^{\,2} \Delta m}{\rho_q v_q A}$$

Sensor area

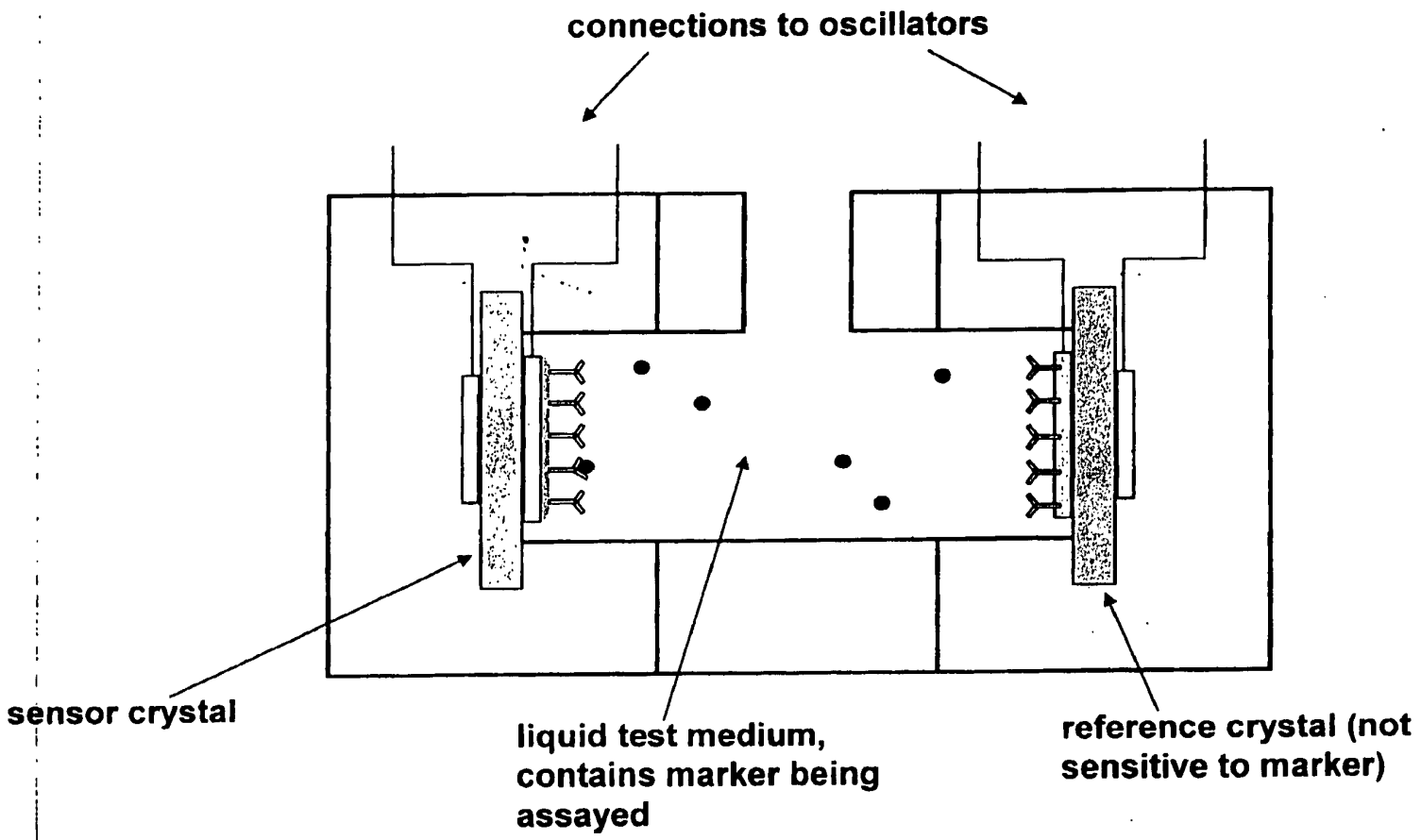
Crystal density

Shear wave velocity

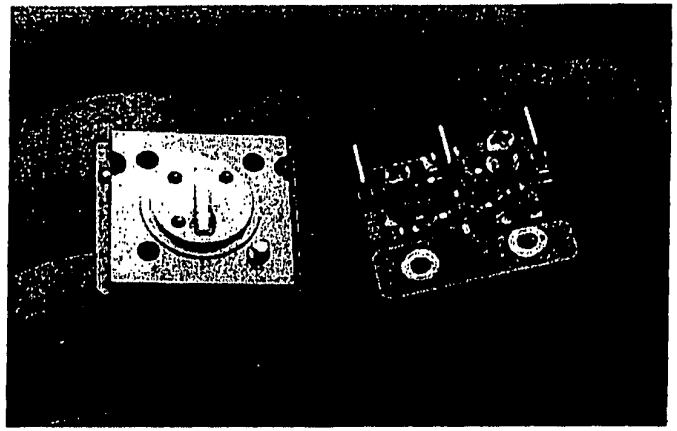
Dual Sensor QCM Setup

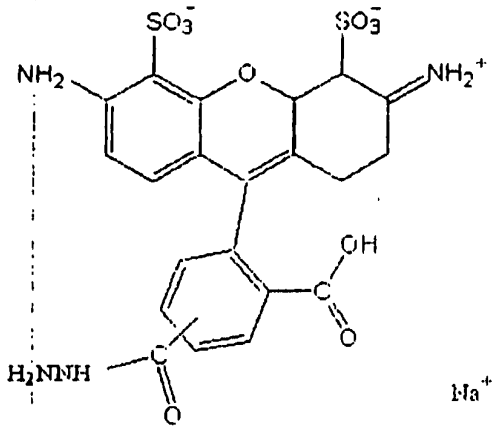


Dual Sensor QCM Flowcell



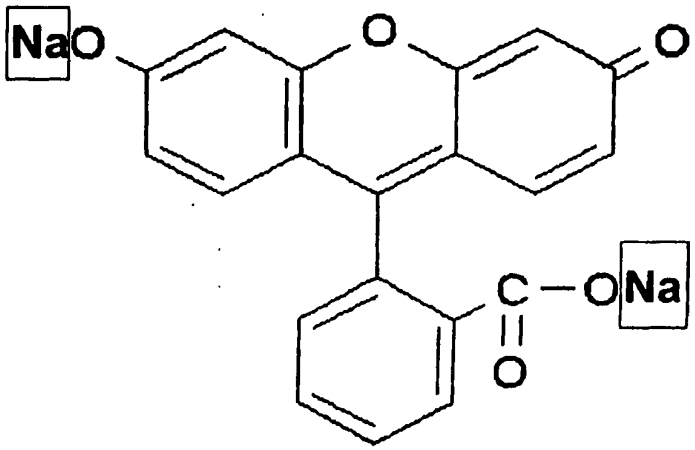
Detection of Small Molecules



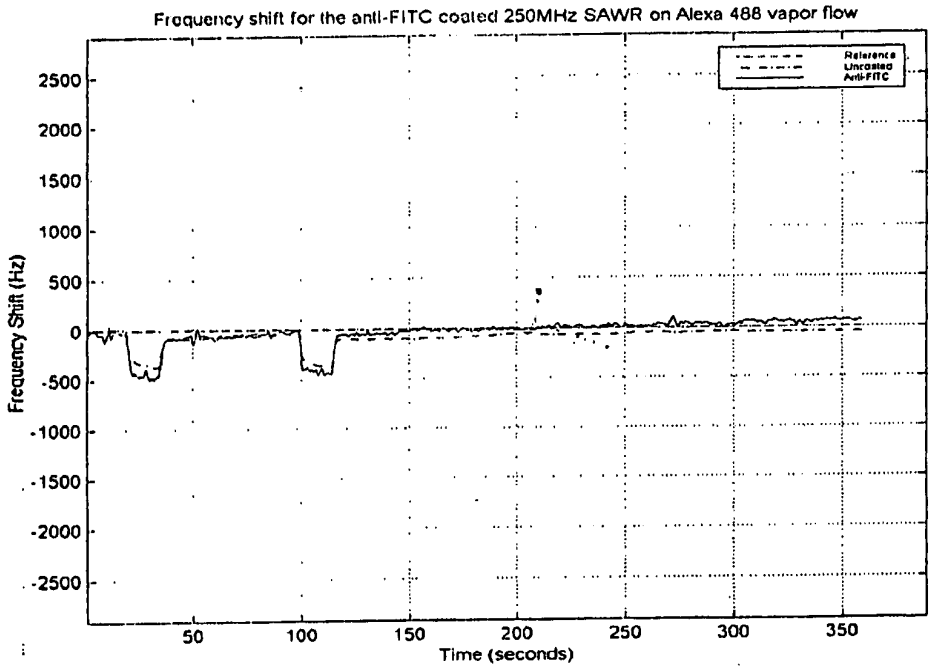


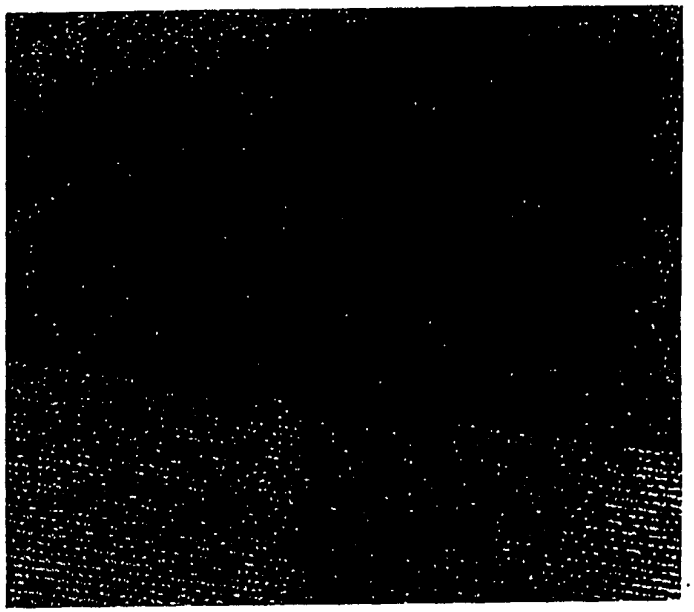
Alexa Flucro 488 Hydrazide, Sodium

Alexa 488 (Molecule A)



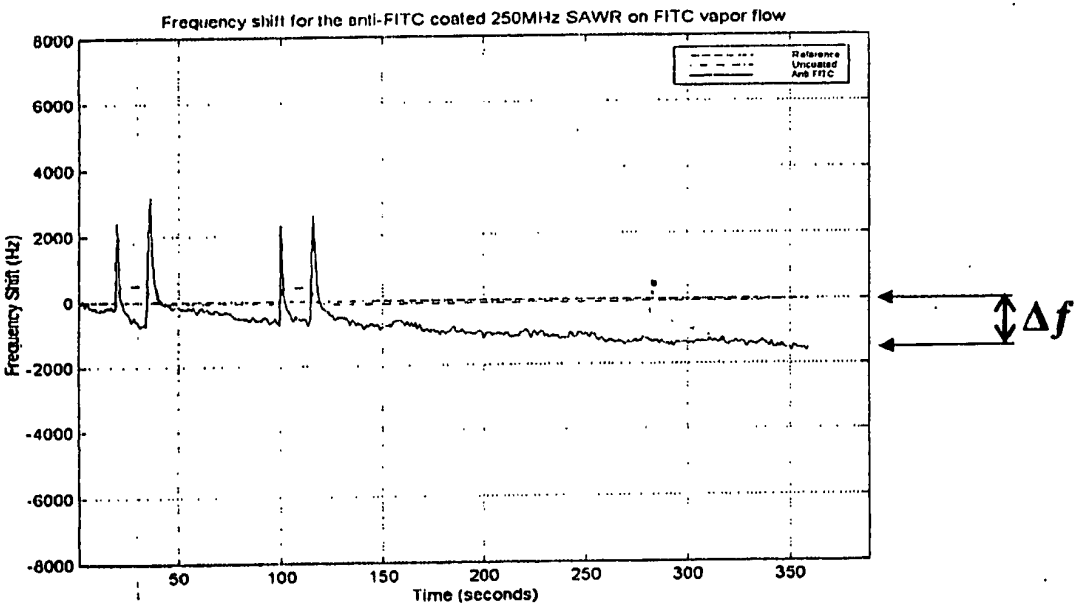
Response of Anti-Uranine Antibody to Alexa 488: 'Molecule A' Not Detected, Successful Negative Control

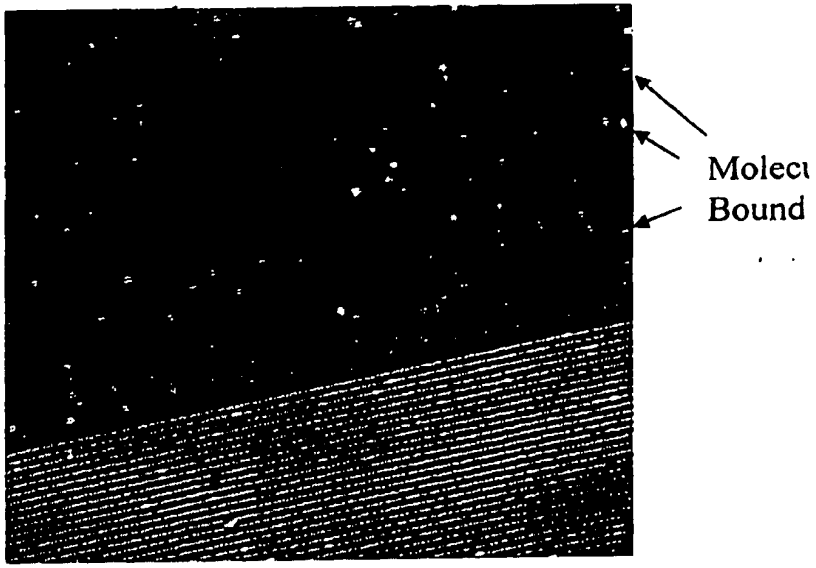




- Response of Anti-FITC Sensor not radically different from uncoated sensor
- The immobilized antibodies have not captured the fluorescent 'Molecule A', (Alexa); the antibodies *discriminate between similar but nonidentical molecules*

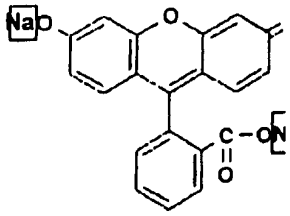
Response of Anti-Uranine Antibody to Uranine: 'Molecule B' is Bound





- **Response of anti-uranine antibody biosensor to uranine is indicative of molecular recognition**

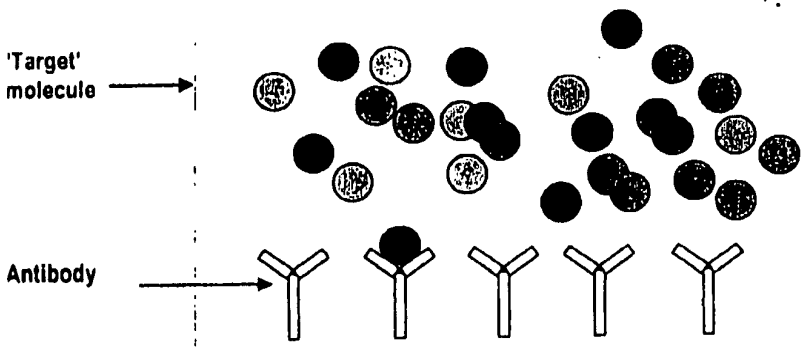
  CLSM image confirms the immobilized antibodies have bound the fluorescent, uranine (Molecule B)

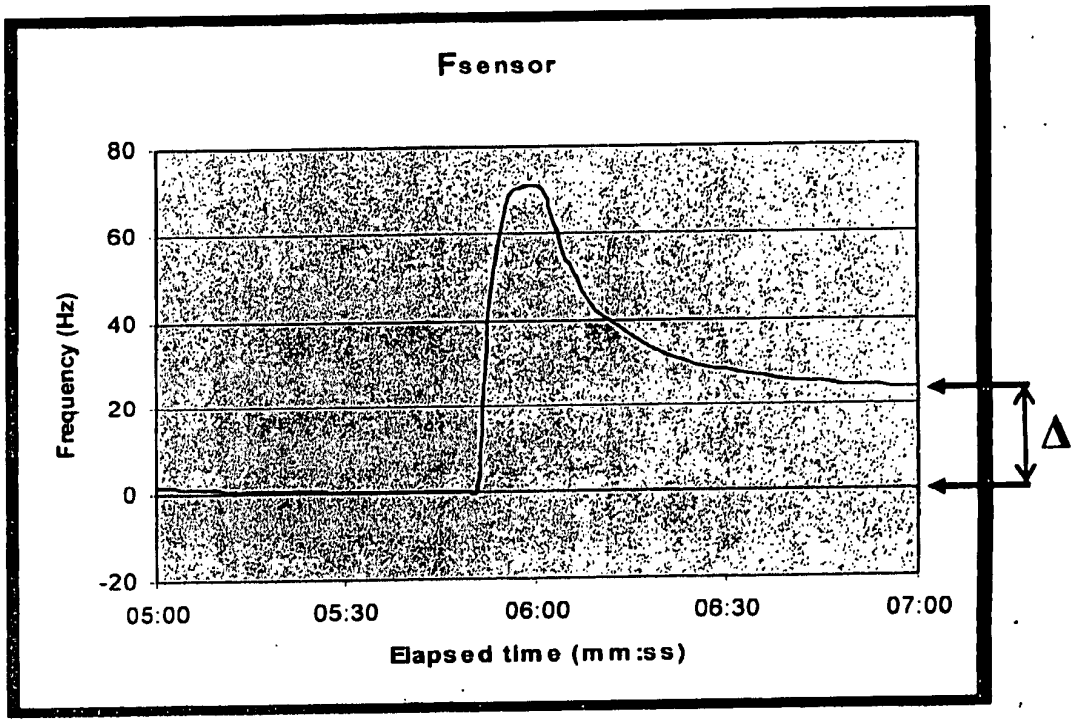


Uranine (Molecule B)

Detecting Uranine in a Slurry of Proteins

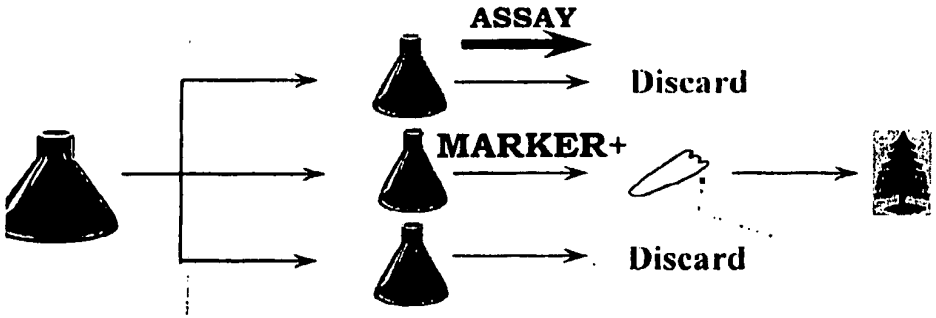
Antibodies Are Molecularly Specific and Bind Targets in a Discriminating Fashion

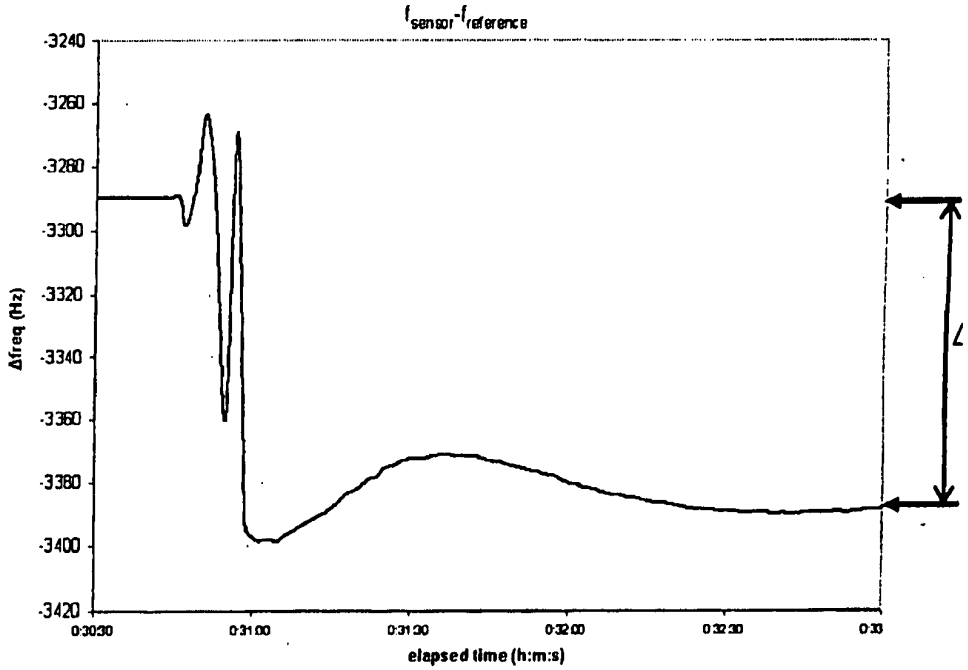




- Nanobiosensors detecting <u>< 1 ppb uranine</u> in a sample of <u>blood serum</u>

Purified Regulatory Protein (Marker), Calmodulin, Detected in Liquid Phase





Nanobiosensor response to addition of 100 μL Calmodulin @ 5 μg/ml concentration→500 ng in solution

Detection limit: with 0.1 Hz noise level, 417 pg

Conclusions

We have improved protocols for increased reliability of protein detection by nanobiosensor

We have achieved detection of purified plant protein (Marker) in liquid phase using the nanobiosensor

We have achieved detection of specific plant protein (Marker) from total plant proteins (thousands of protein types) in liquid phase

We have isolated clones of several new marker proteins (PKL, LEC, ABI3), to be detected in next phase of work

Potential Future Applications

- $ Discriminate between strong and weak cell lines
- $ Bioreactor – production of embryos or biochemicals, feedback control
- $ Biochemical sensor for scaling
- $ Online detection trace chemicals, products of reactions (uranine in serum ⟹ trace chemicals in pulp)
- $ Alternative chemical interactions as detectors, e.g., enzyme-substrate interactions
- $ Seeking funding: DAPRA, NSF, NIH

WHAT IS CLAIMED:

1. An apparatus as described herein and as shown in the Figures, including each and every limitation and embodiment; and

2. A method of operation as described herein and as shown in the Figures, including each and every limitation and embodiment.

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/038021

International filing date: 15 November 2004 (15.11.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/519,800
Filing date: 13 November 2003 (13.11.2003)

Date of receipt at the International Bureau: 02 February 2005 (02.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.